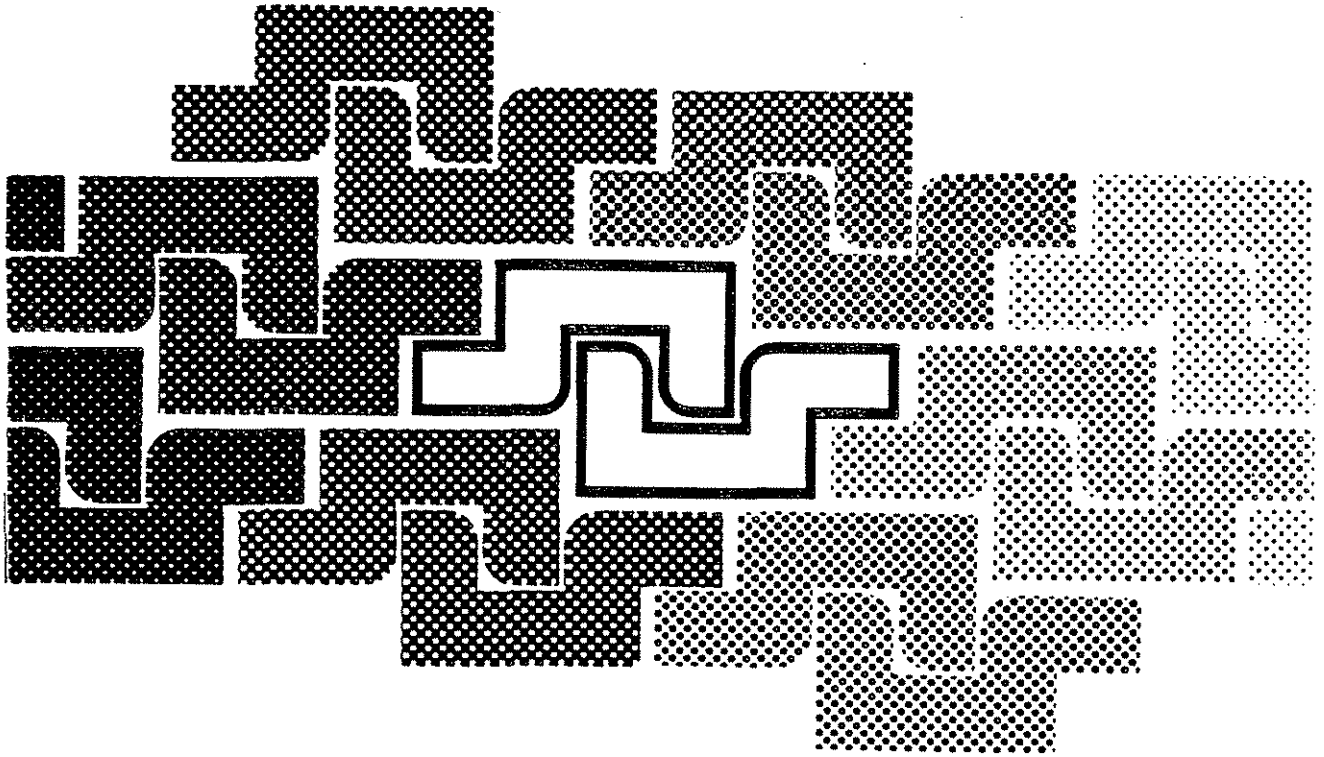

# Modern Concepts in *Penicillium* and *Aspergillus* Classification

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# RIBOSOMAL RNA COMPARISONS AMONG TAXA OF THE TERVERTICILLATE PENICILLIA

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## SUMMARY

Ribosomal RNA sequences were determined for terverticillate Penicillia by the dideoxy nucleotide chain termination method and oligonucleotide primers. The sequences of individual isolates were compared for base differences upon proper alignment. Prior experience in other fungi suggests that a single nucleotide difference in the sequence of two *Penicillium* isolates may indicate that they are not the same species. A second baseline for data interpretation was provided by comparisons involving: *Penicillium*, *Saccharomyces*, and *Urnula*. These intergeneric comparisons revealed >100 base differences.

The maximum number of base differences between species classified in *Penicillium* subgenus *Penicillium* was 33 bases. Our results indicate that *Penicillium aurantiogriseum* NRRL 971, *P. viridicatum* NRRL 963, *P. verucosum* NRRL 965, *P. expansum* NRRL 976, *P. echinulatum* NRRL 1151, *P. hirsutum* NRRL 2032, *P. granulatum* NRRL 2036, and *P. puberulum* NRRL 845 are distinct species. *Penicillium claviforme* NRRL 2031 and *P. clavigerum* NRRL 1003 show a closer relationship to species in subgenus *Penicillium* than to *P. isariiforme* NRRL 2628. Morphological classification schemes that accommodate one or more of the above isolates into a single species are not supported by our results. Three isolates showed no base differences (i.e., *P. puberulum* NRRL 845, *P. resticulosum* NRRL 2021, and *P. camemberti* NRRL 877) and may represent variants of the same species. Ecological and physiological data, as well as secondary metabolite profiles, may be required if one is to distinguish *Penicillium* species by methods other than degree of nucleic acid relatedness.

## INTRODUCTION

Considerable interest and controversy has surrounded taxonomic relationships among *Penicillium* species that produce terverticillate conidiophores (Samson *et al.*, 1976; Pitt, 1979; Frisvad and Filtenborg, 1983). This group includes important food and feed spoilage moulds, pathogens of mature fruits and cereal grains, and "domesticated" isolates used in the fermentation of cheeses or meats (Raper and Thom, 1949; Pitt, 1979; Leistner, 1984). In addition to causing deterioration and quality losses, these moulds may contaminate agricultural products with potent mycotoxins (Frisvad 1986). Correct identification is therefore essential to mycotoxicologists, plant pathologists and food microbiologists. There are considerable problems in attempting to identify terverticillate Penicillia because isolates commonly have characters found in more than one species. In approaching the taxonomy of this group one first must deal with the extensive variation among apparently "healthy isolates" while at the same time recognizing variation associated with strain deterioration in culture (Williams *et al.*, 1985). As with any group of organisms, the views

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of taxonomists differ as to the importance of individual characters in delimiting species (Raper and Thom, 1949; Samson *et al.*, 1976; Pitt, 1979; Frisvad and Filtenborg, 1983). For the terverticillate Penicillia these taxonomic characters include: micromorphology (i.e., conidia, conidiogenous structures), macromorphology (i.e., colony texture and color), physiology (i.e., growth on different substrates at different temperatures and water activities), pathogenicity, and secondary metabolite profiles (SMPs). This research attempts to resolve some of the controversies by comparing of the ribosomal RNA (rRNA) sequences of selected species of terverticillate Penicillia by means of the dideoxy nucleotide chain termination method and oligonucleotide primers. We examined species that are the object of taxonomic disagreement as well as species accepted by all *Penicillium* taxonomists. To provide a baseline for RNA contrasts we examined teleomorph genera *Eupenicillium crustaceum* Ludwig and *Talaromyces helicus*
(Raper & Fennell) Benjamin, known to have a *Penicillium* anamorph state, and two unrelated ascomycetes *Saccharomyces cerevisiae* Hansen and *Urnula craterium* (Schw.) Fr., which presumably are only distantly related to either *Eupenicillium* or *Talaromyces*.

## MATERIAL AND METHODS

The isolates we analyzed for rRNA base sequences are listed in Table 1. Isolates were predominantly from subgenus *Penicillium*. *Eupenicillium crustaceum* also produces terverticillate penicillia (anamorph state = *P. gladioli* McCulloch & Thom), while *Talaromyces helicus* was included as a species representing *Talaromyces*. It produces acerose phialides and typically biverticillate symmetrical penicilli (anamorph state = *P. spirillum* Pitt).

The isolates were grown at 25°C, in 100 ml of YM medium (Wickerham, 1951), on a rotary shaker (200 rpm) for 16-36 hours, until the cultures were in log phase growth. Ribosomal RNA isolation was according to Chirgwin *et al.* (1979), with the exceptions that cells were harvested by filtration, suspended in guanidinium thiocyanate reagent (10 ml/g), and broken in a Braun cell homogenizer with 0.5-mm glass beads. Intact undegraded rRNA, as assessed from denaturing agarose gel electrophoresis, was obtained by this method.

The base sequences of selected regions of the large (25S) and small (18S) subunit rRNA were determined, with specific oligonucleotide primers, by the dideoxy nucleotide chain termination method for RNA sequencing as described by Sanger *et al.* (1977) and Lane *et al.* (1985). Oligonucleotide primer C was purchased from Boehringer-Mannheim (Indianapolis, IN); the other primers were a gift from Carl Woese, University of Illinois. The first base synthesized from the small subunit primer, in relation to the *S. cerevisiae* primary structure (Rubstov *et al.*, 1980), is C, 1627. The first bases synthesized from the large subunit primers, based on *S. cerevisiae* primary structure (Georgiev *et al.*, 1981), are E, 1841 and F, 635. Sulfur-35 labeled nucleotide fragments generated in the chain extension reactions were separated by electrophoresis on 8% acrylamide-8 M urea gels. RNA base sequences were read from autoradiographs of the fixed and dried gels. Sequences with few differences or apparent insertions were rerun side by side on the same gel to verify differences. Some of the sequences were verified by repeating all steps from the beginning. Ribosomal RNA base sequences were aligned manually with a text editor. Alignment was necessary to compare homologous sequences. The data were evaluated with a set of programs that measure simple matching of aligned sequences.

**Table 1. Isolates examined**

*P. atramentosum* Thom NRRL 795 ex type,
*P. puberulum* Bainier NRRL 845 ex neotype,
*P. roqueforti* Thom NRRL 849 ex type,
*P. camemberti* Thom NRRL 877 ex type,
*P. viridicatum* Westling NRRL 963 ex neotype,
*P. verrucosum* Dierckx NRRL 965 ex neotype,
*P. aurantiogriseum* Dierckx NRRL 971 ex neotype,
*P. expansum* Link NRRL 976 ex neotype,
*P. italicum* Wehmer NRRL 983 ex neotype,
*P. clavigerum* Demelius NRRL 1003,
*P. echinulatum* Raper & Thom ex Fassatiová NRRL 1151 ex type,
*P. brevi-compactum* Dierckx NRRL 2011 ex neotype,
*P. resticulosum* Birkinshaw *et al.* NRRL 2021 ex type,
*P. claviforme* Bainier NRRL 2031 ex neotype,
*P. hirsutum* Dierckx NRRL 2032 ex neotype,
*P. granulatum* Bainier NRRL 2036 ex neotype,
*T. helicus* (Raper & Fennell) Benjamin NRRL 2106 ex type,
*P. isariiforme* Stolk & Meyer NRRL 2638 ex type,
*E. crustaceum* Ludwig NRRL 3332 ex type,
*P. arenicola* Chalabuda NRRL 3392 ex type,
*P. fennelliae* Stolk NRRL 3697 ex type,
*P. olsonii* Bainier & Sartory NRRL 13058 ex neotype,
*U. craterium* (Schw.) Fr. SWP-1,
*S. cerevisiae* Hansen NRRL Y-12632.

## RESULTS AND DISCUSSION

Technical limitations, possible artefacts, and difficulties of dideoxy sequencing in ribosomal DNA have been thoroughly considered by Elwood *et al.* (1985), who estimated that 99% sequencing accuracy can be achieved with the dideoxy method and a double-stranded DNA template. Direct ribosomal RNA sequencing with dideoxy methods yields similar accuracy; however, a small percentage of the base positions are impossible to determine because a single stranded template is used. Therefore, we are probably underestimating the total genetic distance between the taxa we have examined. Even so, our sequences are representative of the complete sequences (Lane *et al.*, 1985). The ribosomal RNA base sequences are presented in Figures 1a-c. For *T. helicus* and *U. craterium*, we were unable to read approximately 30% of the sequences located in the region most distal from the primer. To accommodate these species a second matrix was generated based on the readable sequences located proximal to the primer (Fig. 2b).

A baseline for data interpretation was provided by comparisons between species of *Penicillium*, *Eupenicillium*, or *Talaromyces* and two outgroup species, *S. cerevisiae* and *U. craterium*. The relative rates of sequence change for all of the species in this study can be determined by the distance of each strain from the outgroup species. McCarrol *et al.* (1981)recorded an approximate 30% sequence difference between the 18s rRNA of the cellular slime mould *Dictyostelium discoideum* Raper and the ascomycetous yeast *Saccharomyces cerevisiae*. If all of the isolates have been mutating at a nearly constant rate since their divergence from a common ancestor, each strain should be nearly equally separated from the outgroup. Our results show that the outgroup species, *S. cerevisiae* and *U. craterium*, have approximately the same number of base differences with each of the isolates producing a *Penicillium* anamorph (Figs. 2 a-b).

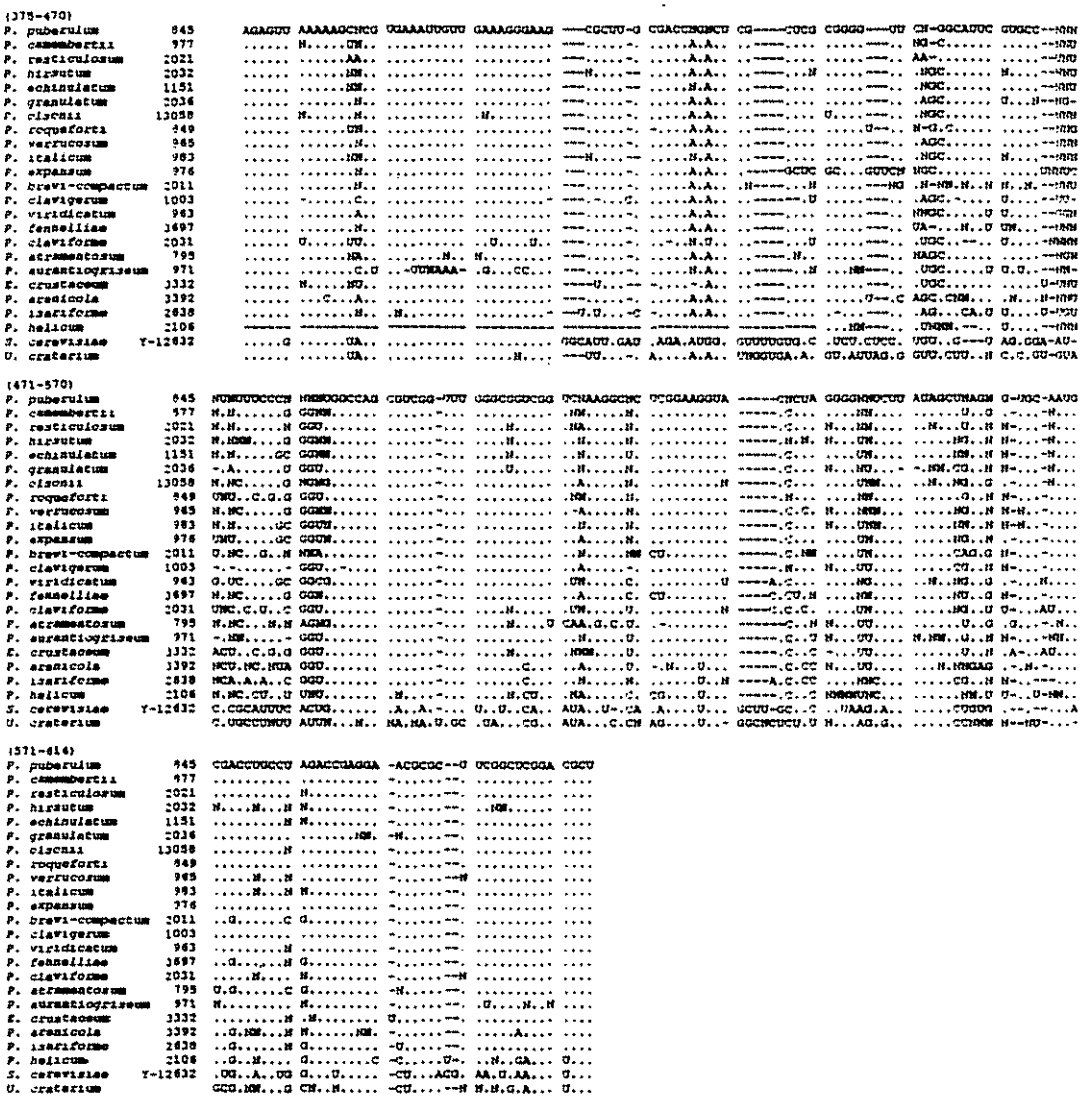


**Figure 1a. Aligned sequences obtained with the F (580r) primer. First base synthesized with this primer corresponds to position 635 of *S. cerevisiae* 25S rRNA. Dots indicate the same base as is found in the first line; dashes indicate missing data (in *U. craterium* and *T. helicum*) or gaps in the sequences; and N indicates that the correct base for that position could not be determined.**

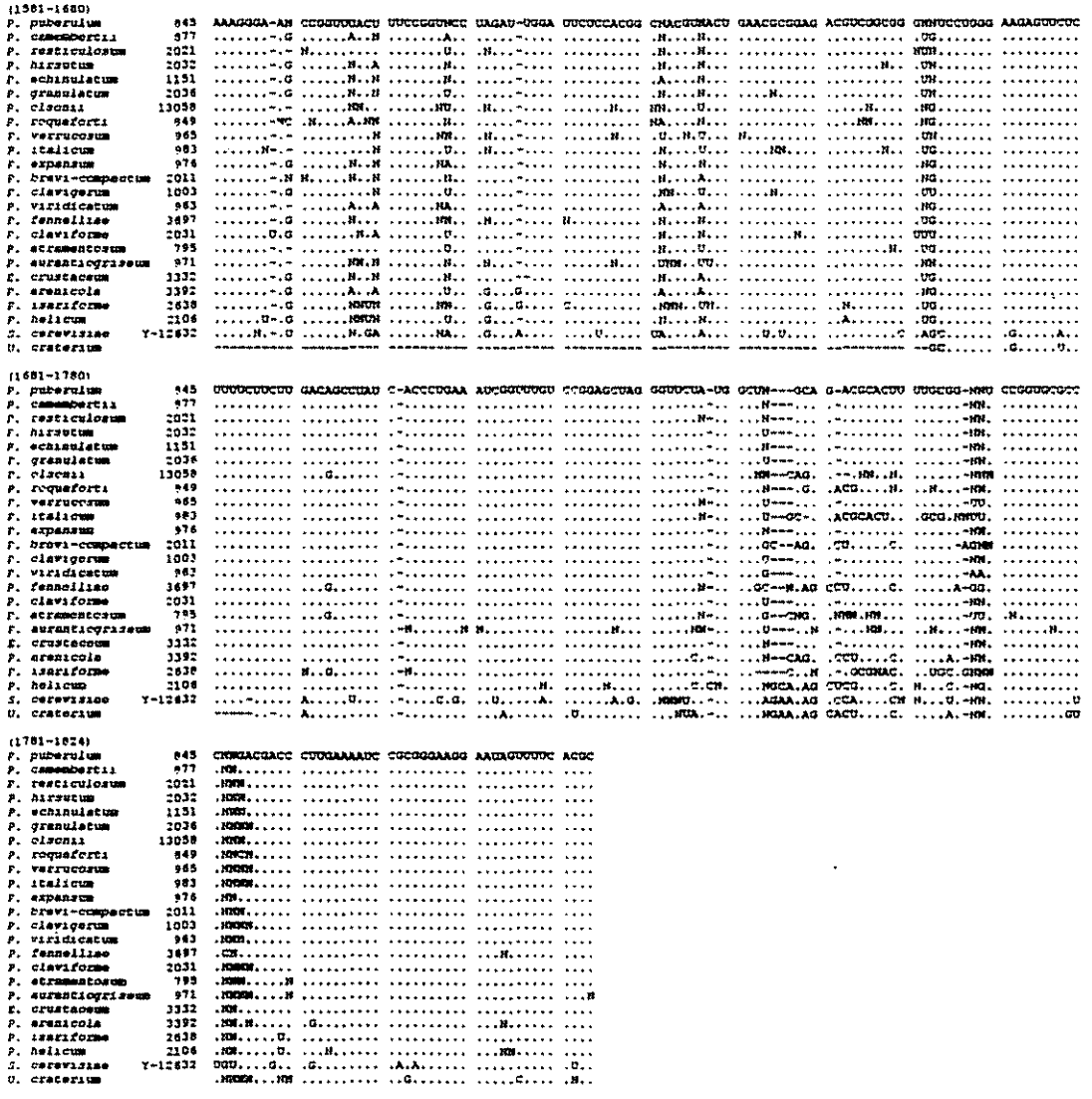


**Figure 1b. Aligned sequences obtained with the E (1611r) primer. The first base synthesized from the primer corresponds to position 1841 of *S. cerevisiae* 25S rRNA. Symbols are the same as Figure 1a.**

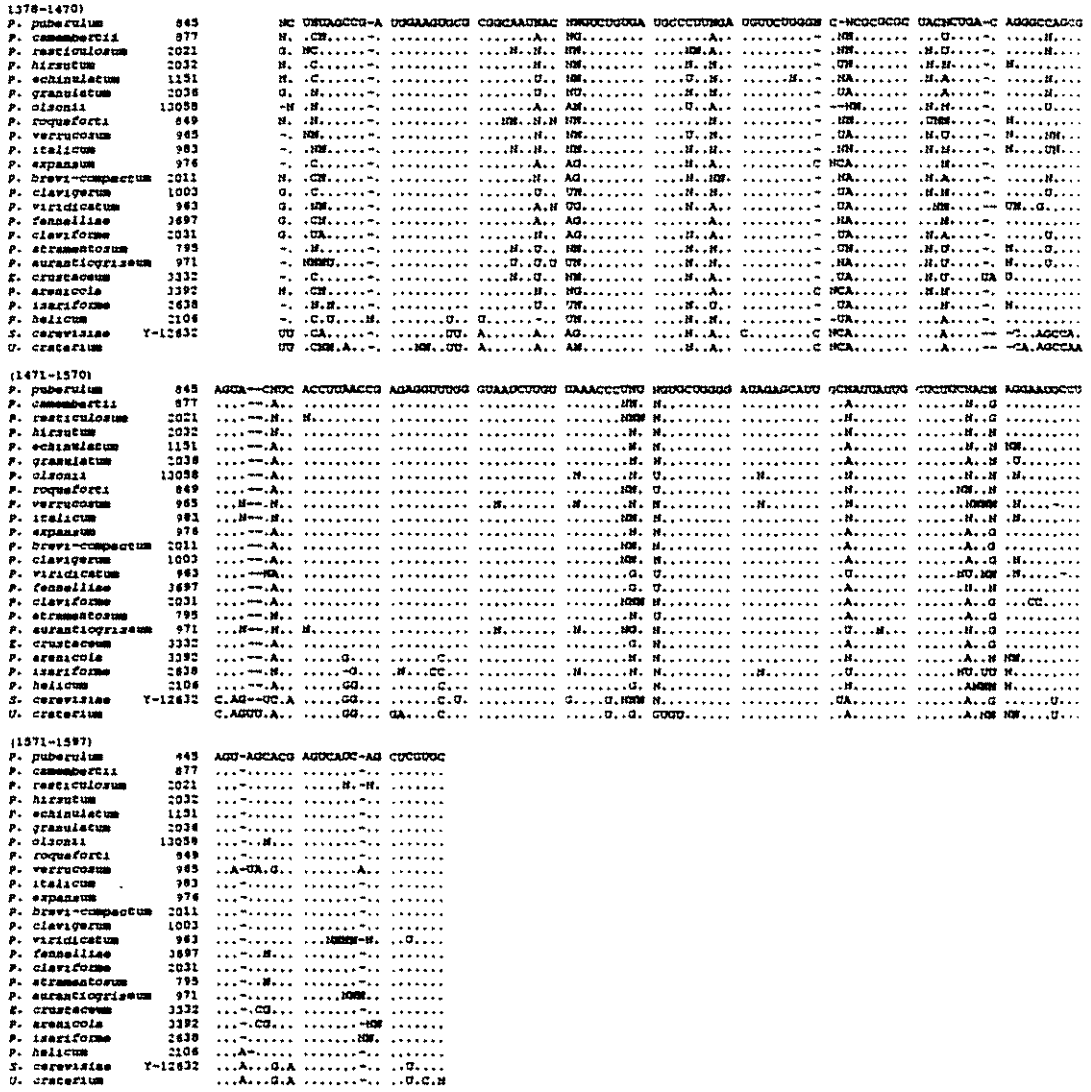
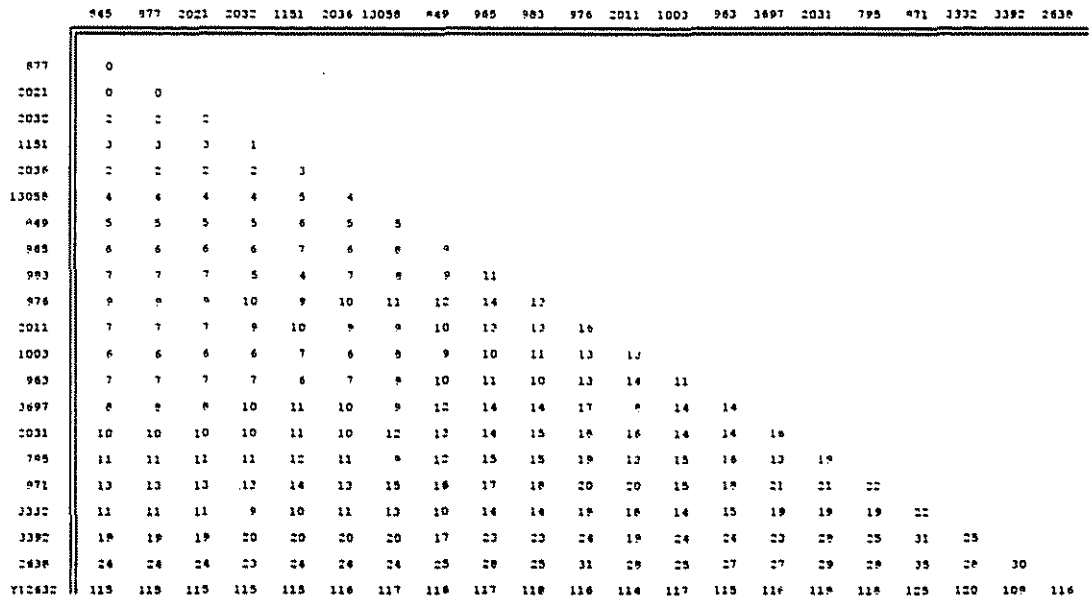


**Figure 1c. Aligned sequences obtained with the C primer. The first base synthesized corresponds to position 1627 of the *S. cerevisiae* 18S rRNA. Symbols are the same as in Figure 1a.**



| | 845 | 877 | 2021 | 2032 | 1151 | 2036 | 13058 | 449 | 965 | 983 | 976 | 2011 | 1003 | 963 | 3697 | 2031 | 795 | 971 | 3332 | 3392 | 2638 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 877 | 0 | | | | | | | | | | | | | | | | | | | | |
| 2021 | 0 | 0 | | | | | | | | | | | | | | | | | | | |
| 2032 | 2 | 2 | 2 | | | | | | | | | | | | | | | | | | |
| 1151 | 3 | 3 | 3 | 1 | | | | | | | | | | | | | | | | | |
| 2036 | 2 | 2 | 2 | 2 | 3 | | | | | | | | | | | | | | | | |
| 13058 | 4 | 4 | 4 | 4 | 5 | 4 | | | | | | | | | | | | | | | |
| 449 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | | | | | | | | | | | | | | |
| 965 | 6 | 6 | 6 | 6 | 7 | 6 | 6 | 6 | | | | | | | | | | | | | |
| 983 | 7 | 7 | 7 | 5 | 4 | 7 | 6 | 9 | 11 | | | | | | | | | | | | |
| 976 | 9 | 9 | 9 | 10 | 9 | 10 | 11 | 12 | 14 | 12 | | | | | | | | | | | |
| 2011 | 7 | 7 | 7 | 9 | 10 | 9 | 9 | 10 | 12 | 13 | 16 | | | | | | | | | | |
| 1003 | 6 | 6 | 6 | 6 | 7 | 6 | 6 | 9 | 10 | 11 | 13 | 13 | | | | | | | | | |
| 963 | 7 | 7 | 7 | 7 | 6 | 7 | 6 | 10 | 11 | 10 | 13 | 14 | 11 | | | | | | | | |
| 3697 | 8 | 8 | 8 | 10 | 11 | 10 | 9 | 12 | 14 | 14 | 17 | 8 | 14 | 14 | | | | | | | |
| 2031 | 10 | 10 | 10 | 10 | 11 | 10 | 12 | 13 | 14 | 15 | 18 | 16 | 14 | 14 | 16 | | | | | | |
| 795 | 11 | 11 | 11 | 11 | 12 | 11 | 9 | 12 | 15 | 15 | 19 | 12 | 15 | 16 | 13 | 19 | | | | | |
| 971 | 13 | 13 | 13 | 12 | 14 | 13 | 15 | 16 | 17 | 18 | 20 | 20 | 15 | 18 | 21 | 21 | 22 | | | | |
| 3332 | 11 | 11 | 11 | 9 | 10 | 11 | 13 | 10 | 14 | 14 | 19 | 18 | 14 | 15 | 19 | 19 | 19 | 22 | | | |
| 3392 | 19 | 19 | 19 | 20 | 20 | 20 | 20 | 17 | 23 | 23 | 24 | 19 | 24 | 24 | 23 | 23 | 25 | 31 | 25 | | |
| 2638 | 24 | 24 | 24 | 23 | 24 | 24 | 24 | 25 | 28 | 25 | 31 | 28 | 25 | 27 | 27 | 29 | 28 | 35 | 28 | 30 | |
| Y12632 | 115 | 115 | 115 | 115 | 115 | 116 | 117 | 116 | 117 | 118 | 116 | 114 | 117 | 115 | 116 | 118 | 118 | 125 | 120 | 109 | 116 |

**Figure 2a. Matrix of base differences between the *Penicillium* species and *S. cerevisiae*. Gaps in sequences are counted as mismatches to any base present. Base positions for which the correct base could not be determined for one or more strains were excluded from the calculation. Total sequence length analysed is 708 positions.**

| | 845 | 3332 | 3697 | 2638 | 2106 | URNULA |
|---|---|---|---|---|---|---|
| 3332 | 6 | | | | | |
| 3697 | 8 | 14 | | | | |
| 2638 | 16 | 22 | 19 | | | |
| 2106 | 27 | 31 | 26 | 27 | | |
| URNULA | 85 | 88 | 81 | 85 | 75 | |
| Y-12632 | 80 | 84 | 83 | 79 | 72 | 75 |

**Figure 2b. Matrix of base differences between *Eupenicillium*, *Talaromyces*, *Urnula*, and *Saccharomyces*. Results were calculated as in figure 2a. Total length of sequence examined, 555 bases.**

It was recently proposed that the teleomorph genera *Eupenicillium* and *Talaromyces* with *Penicillium* anamorphs, represent separate lines of evolution involving cleistothecial Ascomycetes (Malloch, 1985). Malloch theorized that species in subgenus *Biverticillium* are more closely related to *Talaromyces* since they can degrade cellulose and were probably derived from species colonizing decayed wood such as *Trichoma* in the subfamily Trichocomoideae. Malloch (1985) classified *Penicillium* anamorphs with a marked affinity for starchy or oily substrates in the subfamily Dichlaenoideae. The latter would encompass those species in subgenus *Penicillium* that are commonly isolated from agricultural products (Pitt, 1979). *Eupenicillium crustaceum* and *T. helicum* differed by 31 bases in the abbreviated sequence length. Because the non-readable portion of the sequence contained numerous base differences in other species, we suggest that these genera could have as many as 40-45 different bases over the entire sequence length. *Urnula craterium* and *S. cerevisiae* differed from *E. crustaceum* and *T. helicum* by 72-88 bases in the abbreviated sequence length (Fig. 2b). At the same time, *U. craterium* and *S. cerevisiae* differ from each other by 75 bases (Fig. 2b). These results suggest that the two major teleomorph genera having *Penicillium* anamorphs can be traced to the same branch in Ascomycete evolution. If the two genera had entirely independent origins we would have expected a number of base differences equivalent to that recorded in contrasts involving *Urnula* and/or *Saccharomyces*.

All but three of the isolates we examined differed by one or more bases and may represent distinct species (Fig. 2a). Strains having no base differences (i.e., *P. puberulum* NRRL 845, *P. resticulosum* NRRL 2021, and *P. camemberti* NRRL 877) may represent variants of the same species. In heterothallic yeasts, isolates of a sexually reproducing species have an identical ribosomal RNA sequence, but isolates identified as siblings, on the basis of mating reactions and DNA complementarity, differ by as few as 2 and up to 7 base substitutions. Six isolates of *S. cerevisiae* representing isolates from different sources had identical base sequences (S. Peterson and C. P. Kurtzman, unpublished). If these data are representative of other fungi, a single nucleotide difference in the sequence of two *Penicillium* isolates suggests that they are not the same species. This information will aid in the resolution of several questions about taxonomic and evolutionary relationships among the isolates of terverticillate Penicillia that we sequenced. Our results indicate that *P. verrucosum* NRRL 965, *P. viridicatum* NRRL 963, *P. aurantiogriseum* NRRL 971, *P. hirsutum* NRRL 2032, and *P. puberulum* NRRL 845 (all ex neotype cultures) represent distinct species. Samson *et al.* (1976) accommodated these and several other species in *P. verrucosum* Dierckx. At that time, this was justified primarily on the basis of morphological characteristics of the conidiogenous structures (e.g., fasciculate Penicillia with two-staged, sometimes three-staged branched, rough-walled conidiophores and globose to subglobose, smooth to slightly rough-walled conidia). Samson *et al.* (1976) recognized strain NRRL 965 as the neotype culture of *P. verrucosum* and included this strain in *P. verrucosum* var. *verrucosum* Samson *et al.*, along with strain NRRL 963 (= *P. viridicatum* Westling). Pitt (1979) retained *P. verrucosum* as a species and distinguished it from *P. viridicatum*. Frisvad and Filtenborg (1983) used SMPs to place these and other isolates of terverticillate Penicillia into species and provisional nonbotanical subgroups. The authors proposed that SMPs, combined with recognizable microscopic and simple physiological criteria, should be one of the bases for the establishment of a new classification system of the terverticillate Penicillia. It was the authors' intent to allow mycologists time to consider these experimental groupings before formally erecting new varieties or species. Stolk and Samson (1985), citing "practical reasons" and the SMPs of Frisvad and Filtenborg (1983), decided to reverse their earlier classification scheme

(Samson *et al.*, 1976) and list these Penicillia as species. Our results provide evidence that these distinct *Penicillium* chemotypes represent distinct species.

*P. puberulum* NRRL 845 and *P. camemberti* NRRL 877, ex type, showed identical base sequences. At the same time, *P. camemberti* NRRL 877 and *P. aurantiogriseum* NRRL 971, ex neotype, differed by 15 bases. This result does not support the hypothesis that *P. aurantiogriseum* is the wild-type ancestor of the domesticated cheese mould *P. camemberti* as suggested by Samson (1985). Cruickshank and Pitt (1987) reported that *P. puberulum* (NRRL 2040, ex neotype) produced zymograms, suggesting synonomy with *P. aurantiogriseum*, but our data indicating 13 base substitutions argues strongly against this (Table 2a). The authors considered *P. commune* Thom NRRL 890a ex neotype to be incorrectly placed in *P. puberulum* by Pitt (1979). The rRNA base sequences of this *P. commune* strain were not examined and, therefore, cannot address the question of whether *P. commune*, like *P. puberulum*, should also be recognized as a synonym of *P. camemberti*.

"Domesticated" Penicillia used in food fermentations were derived from naturally occurring "wild" species (Samson, 1985) but *Penicillium* taxonomists may disagree as to which species represent the "wild" progenitor (Polonelli *et al.*, 1987). Frisvad and Filtenborg (1983) established the chemotype *P. camemberti* II to include species formerly classified in *P. commune* Thom. *Penicillium camemberti* was recognized as a domesticated form of *P. commune*, the wild form occurring in nature (Polonelli *et al.*, 1987). The search for a wild-type strain of *P. camemberti* is now answered with the type strain of *P. puberulum* isolated from corn. Because *P. camemberti* was described in 1906, while *P. puberulum* was described in 1907, the combination *P. puberulum* var. *camemberti* would be unacceptable according to the rules of nomenclature. Our data do not support placement of *P. puberulum* in synonomy with *P. aurantiogriseum* (Samson *et al.*, 1976) because the neotype isolates differed by 15 bases. Raper and Thom (1949) noted that *P. puberulum* NRRL 1889 and *P. puberulum* NRRL 845 came from the same original source, Thom No. 4876.20, a strain isolated from *Zea mays* L. and the basis of a classic paper on penicillic acid formation by Alsberg and Black (1913). Strain NRRL 845, received by C. Thom in 1935, had changed in cultural appearance, becoming more loose in texture and lighter sporing, and resembled *P. commune*. Thom and Raper (1949) were not certain of the taxonomic position of *P. puberulum*. The production of velvety colonies led Thom (1930) to place *P. puberulum* in the Asymmetrica-velutina section, but Thom and Raper (1949) noted the development of limited fasiculate structures in older colonies, and other characters suggested a relationship to *Penicillium cyclopium* series in the Asymmetrica-fasciculata section.

*P. resticulosum* was originally isolated as a culture contaminant in Birkinshaw's laboratory (Raper and Thom, 1949). *P. puberulum* NRRL 845 and *P. resticulosum* NRRL 2021 have identical base sequences. *P. puberulum* NRRL 845 is a loose-textured, lightly sporulating, cultural variant of isolate NRRL 1889. Both NRRL 845 and NRRL 1889 were extensively investigated in Birkinshaw's laboratory and it is interesting to speculate that NRRL 2021 represents another cultural variant of *P. puberulum* NRRL 1889. *P. puberulum* is reported to form limited fasiculate structures suggesting a relationship to the *P. cyclopium* series in the Asymmetrica-fasciculata section (Raper and Thom, 1949).

Samson *et al.* (1976) considered *P. resticulosum* to be a floccose variant of *P. expansum*. This is not supported by our results, which show that *P. expansum* and *P. resticulosum* differed by 9 bases. Pitt (1979) suggested that *P. resticulosum* was a distinct, rare species, but reduced it to synonymy with *P. expansum* (Cruickshank and Pitt, 1987). It is important to recognize that the three isolates with identical base sequences (i.e., *P. puberulum* NRRL 845, *P. resticulosum* NRRL 2021, *P. camemberti* NRRL 877) show identical numbers of

different bases in contrast with other *Penicillium* isolates (Fig. 2a). The observation that some species assigned by Raper and Thom (1949) to the sections *Asymmetrica* subsect. *Funiculosa* and subsect. *Lanata* represent cultural variants of isolates classified in subsections *Velutina* or *Fasiculata* (Samson *et al.*, 1976; Pitt, 1979) is consistent with our findings.

Frisvad and Filtenborg (1983) proposed that *P. arenicola*, *P. fennelliae* and *P. olsonii*, species that Pitt (1979) included in subgen. *Penicillium*, were taxonomically distinct from "true species" of terverticillate Penicillia. We could not separate *P. fennelliae* or *P. olsonii* from the more typical species belonging to subgen. *Penicillium* on the basis of substantial differences in rRNA base sequences. *P. arenicola* showed a consistent pattern of higher numbers of base differences when contrasted with the other terverticillate Penicillia. Stolk and Samson (1985) noted that *P. arenicola* is not a typical *Penicillium*, but retained it in *Penicillium* in agreement with Pitt (1979). Pitt (1979) included *P. fennelliae* in subgen. *Penicillium* on the basis of the orginal illustrations, but noted that the isolates he examined produced predominantly biverticillate Penicillia. Our results indicate a closer relationship to species in subgen. *Penicillium* than to *P. isariiforme* in subgenus *Biverticillium*.

Raper and Thom (1949) classified *P. olsonii* in sect. *Biverticillata-Symmetrica*. Our results suggest that *P. olsonii* NRRL 13058 (ex neotype) is more closely aligned with species classified in subgen. *Penicillium* (Pitt, 1979).

*P. claviforme* NRRL 2031 (= *P. vulpinum* Cooke & Massee) Seifert & Samson) and *P. clavigerum* NRRL 1003 share more bases in common (14 base differences) than either taxon does with *P. isariiforme* NRRL 2628 (28 and 24 differences, respectively). Raper and Thom (1949) placed *P. claviforme* and *P. clavigerum* in subsection *Fasciculata* because they form coremia, but Pitt (1979) classified these species in subgen. *Biverticillium* with *P. clavigerum* being placed in synonymy with *P. duclauxii*. Frisvad and Filtenborg (1983) distinguished *P. isariiforme* on the basis of SMPs and strongly yellow-colored mycelium, agreeing with its placement in subgen. *Biverticillium*, with *P. claviforme* and *P. clavigerum* remaining in subgen. *Penicillium*. Our base se quence data supports their classification.

*P. cyclopium* var. *echinulatum* Raper & Thom was not validly published and Fassatiová (1977) validated and raised it to species status. Our results confirm that *P. echinulatum* NRRL 1151 and *P. aurantiogriseum* NRRL 971 (= *P. cyclopium*) are distinct species. *P. granulatum* Bainier (= *P. glandicola* (Oud.) Seifert & Samson) is recognized as sharing characteristics in common with *P. verrucosum* and *P. brevicompactum* (Pitt, 1979) but our results indicate that *P. granulatum* NRRL 2036 shares more bases with *P. puberulum*, *P. olsonii*, and *P. hirsutum*.

Classification schemes which rely on physiological characters (e.g., growth rates, toxin production) as well as morphological characters are supported by our results. Ecological and physiological data as well as SMPs are required if one is to distinguish *Penicillium* species by methods other than degree of nucleic acid relatedness. Wicklow (1985) observed that physiological attributes (Pitt, 1979), and SMPs (Frisvad *et al.*, 1983) are ecologically relevant characters that define the fungal niche. The fundamental niche of a fungus can be defined in the laboratory by careful control of climate, substrate chemistry, and interacting organisms (McNaughton, 1981). If the niche parameters of two isolates are distinct, it is likely they occupy different niches and would represent different species.

Williams *et al.* (1985) suggest that the considerable variation we find in subgen. *Penicillium* may result from the "rapid adaptation of a relatively few ancient species to take advantage of the many new nutritional niches provided by man during the few millennia of his agricultural activity." An example of this is demonstrated by our results showing that the domesticated white cheese mould *P. camemberti* has no base differences with the

naturally occurring wild species *P. puberulum*. At the same time, those terverticillate Penicillia whose sequences differ by one or more bases represent species that predate human agriculture.

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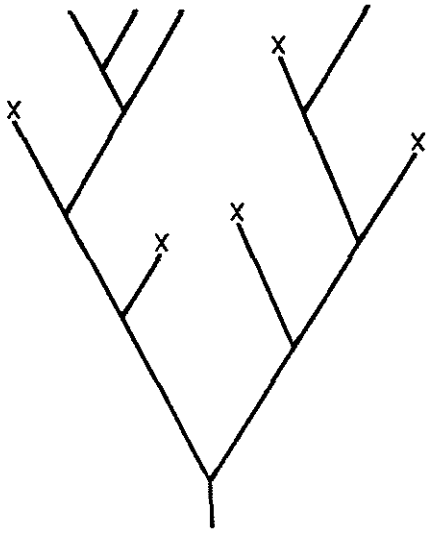
## DIALOGUE FOLLOWING DR. PETERSON'S PRESENTATION

GAMS: I would like to ask Dr. Taylor and Dr. Peterson where they would draw the line to distinguish species using your techniques. Are your techniques sensitive enough to really distinguish species?



TAYLOR: This figure gives an indication of what molecular techniques can and cannot tell us. Molecular techniques can give us this whole story if we do enough work. In this diagram, we see species diverging and becoming extinct, diverging and becoming extinct, as we pass through time. Finally, at the bottom, we see the species on the left is

quite distinct and no one has any problem recognizing it. The three species on the right, however, remain close together, and are difficult to distinguish, no matter what methods are used. With anamorphic genera there will always be the problem that closely related species are going to be difficult to distinguish. If these taxa are important, such as being mycotoxin producers, then they will be distinguished for practical reasons. If not, if nobody cares about them, they will be lumped together.

PETERSON: I agree with Dr. Taylor. Ribosomal RNA shows us the phylogeny but doesn't give us ability to assign a taxonomic level to a taxon. So, we're seeing a pattern of descent and it's still a philosophical decision whether something is a species or a variety.

GAMS: You said you could not distinguish some of the terverticillate Penicillia at all, but in your diagrams you show differences of two or three base changes. Is this not sufficient?

PETERSON: Our work with heterothallic species of yeasts, in which we do have a biological species concept, is the only way we have of calibrating what these base changes mean taxonomically. In sexually reproducing species, up to two base changes may exist in a single species. If there were fifteen bases differences, the case for considering these distinct species is overwhelming.

PITT: The work that is done with yeasts is fascinating, but it is irrelevant to the kind of fungi we are considering here. It's impossible to relate a yeast species to a *Penicillium* or *Aspergillus* species. The genome sizes are so different. We don't know anything about the mating patterns in these moulds, of course. I think you should ask quite a different question. Can you take ten isolates of *P. aurantiogriseum* and ten of *P. commune*, which people in this area consider to be separate species, and make the distinction between intraspecific variation in the parameter you are measuring, and the variation between species?

PETERSON: The point is well taken. We have been planning to take this approach in our laboratory. We had planned to use *P. chrysogenum* rather than *P. commune*. This needs to be done.

PITT: When you do this work, please have your isolates checked by at least one other taxonomist.